

OBSERVATIONS ON THE SYMBIOSIS OF THE SEA ANEMONE STOICHACTIS AND THE POMACENTRID FISH, AMPHIPRION PERCULA¹

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The partnership between certain tropical damselfishes and sea anemones has excited the interest of students of natural history for almost a century. The most significant investigations of the symbiosis have been those of Sluiter (1888), Verwey (1930) and Gohar (1948), who have given us some knowledge of the ecology and behavioral characteristics of the animals. In 1947 Gudger reviewed all the observations that had been made up to that time, and in 1950 Baerends first speculated about the possible role of releasers in the maintenance of the association.

However, this symbiosis, like many others, still poses many unanswered questions. The physiological and behavioral mechanisms which maintain the animals in partnership have not been investigated with present-day techniques.

It has not been clear whether the fish responds to chemical, tactile or visual stimuli from the host, nor whether the behavior of the anemone is affected by stimuli from the fish. The mechanism whereby the fish is protected from the nematocysts of the host has been a mystery. In spite of the fact that it is generally supposed that nematocysts are not under nervous control but that they fire off independently upon adequate stimulation, several investigators have speculated that in such partnerships the presence of the fish in some way causes the coelenterate host to put its nematocysts "out of action" (Baerends, 1957, p. 262). The question remains whether the fish simply fails to provide adequate stimuli to discharge the nematocysts, or whether a factor is produced by the fish which markedly raises the threshold of discharge of the nematocysts and thus affords protection. Finally, it remains to be determined whether or not the fish is immune to the poison of the nematocysts.

Recently at Marineland of the Pacific it became possible to investigate the partnership between *Amphiprion percula* (Lacépède) and the giant anemone *Stoichactis* (Fig. 1). We directed our attention primarily to the physiological and behavioral mechanisms involved in the protection of the fish against the nematocysts of its host and in the course of the work were able to re-examine and re-establish some of the observations of Verwey and Gohar.

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FIGURE 1. The anemone *Stoichactis* and two partner *Amphiprion percula*. Photographed in the exhibition aquarium at Marineland of the Pacific. Approximately $\times \frac{1}{6}$.

MATERIAL AND METHODS

Experimental fish were obtained on the reefs near Nasugbu, Batangas Province, Luzon, in the Philippine Islands by commercial collectors. Our single specimen of the host anemone was taken at the same locality. Verwey (1930) describes an anemone, probably identical with ours, from Batavia Bay, Java (Anemone 1, Plate XV, Fig. 2) which he says is colonized in nature by *Amphiprion percula* alone.² Ours was provided by the collectors specifically as the host of *A. percula*. At this writing it is still alive at Marineland. We believe it to be *Stoichactis kenti* (Haddon and Shackleton) although precise identification will not be possible until examination of the internal anatomy can be made after the animal is preserved.

The anemone was received at Marineland in January of 1957, and our experiments were started on September 3, 1957. Thus the animal was acclimated to Marineland sea water for a period of somewhat over 8 months. During the period of our observations it was maintained at 25° C. in a 60-gallon "photographic" redwood aquarium, which was so constructed that a sheet of glass could be inserted to isolate fishes from the anemone when desired.

We received a total of thirteen specimens of *Amphiprion percula*. The previous

² In the aquarium at Batavia this anemone was readily occupied by *Amphiprion akallopisus* and *A. polynemus*, in addition to *A. percula*.

history of these fish is totally unknown to us. Probably some or all of the animals were collected from anemones. However, according to Dr. José Montilla of the Division of Marine Fisheries in Manila, this species of *Amphiprion* does not always live in association with anemones in the Philippines; hence some of our experimental fish may have been free-living. Also, it is known that *A. percula* lives in association with at least two species of anemones (Verwey, 1930). Therefore the host habit of any that may have been commensal is also unknown to us.

Prior to the experiments, two fish, A and B, were kept in partnership with the anemone for several weeks. These gave us controls which we knew were "acclimated" to the anemone. Nine other fish (C to K) had been isolated from any possible sensory contact with an anemone host for a period of not less than six weeks. In the following experiments these are spoken of as "unacclimated" fish.

Two other *A. percula* (L and M) which had occasionally been put in with the anemone for exhibition purposes prior to our experiments were also used. One of these (L) was the largest animal in our sample, measuring 65 mm. standard length. Fish M, a small animal, was sacrificed in a physiological experiment.

The age and sex of our *Amphiprion percula* were not determined.

A single adult specimen (56 mm. standard length) of *Amphiprion frenatus* Brevoort was available for specificity studies. This fish had lived in the exhibition aquarium with the anemone for the eight months prior to our experiments but was never observed to enter it.

The most careful precautions were taken to maintain all glassware, forceps, scissors, dip nets, and other tools free from contamination with organic materials, because of the well-known sensitivity of nematocysts to such substances. All items employed in the manipulation of fishes or isolated anemone tentacles were scrubbed with detergent, washed in distilled water, dipped in ether-alcohol and allowed to dry without contact. Fish to be sacrificed were dissected with clean instruments in clean Petri dishes. Whenever possible, experimental fish were not handled at all but were trapped in the aquaria with clean 500-cc. beakers. When it was necessary to use a nylon dip net, the net was first boiled and rinsed.

Experiments on the discharge of nematocysts from isolated tentacles were conducted in clean watch glasses. A new tentacle was prepared for each test. Tentacles were isolated by clipping them off at the base with clean, fine-tipped scissors. They were stimulated mechanically with a clean glass rod drawn to a fine point, and electrically stimulated with a platinum wire-glass electrode drawn to a fine capillary point. The electrode was connected in a circuit with a standard inductorium, key, and a 6-volt dry cell. A small piece of aluminum foil dipped into the sea water in the watch glass served as the other electrode. Between each experiment, the watch glass, the glass rods, the platinum wire, and the capillary tube were washed with ether-alcohol; the capillary tube was refilled with clean sea water, and the aluminum electrode was replaced.

In certain experiments, one-cm. cubes of plastic sponge were used. These were cut from the center of a new commercial sponge by use of a clean single-edge razor blade.

OBSERVATIONS ON THE "PROCESS OF ACCLIMATION"

It has been observed (Gohar, 1948) that the acclimation of an *Amphiprion* to an anemone may take a considerable length of time. The details of this acclima-

tion remain virtually unknown. We felt that careful observation of this process might give us insight into the mechanism which protects the fish from nematocyst discharge.

Accordingly, a series of nine experiments were performed in which we introduced individual unacclimated *Amphiprion percula* into the observation tank with the anemone. These tests revealed a fairly stereotyped series of events which terminated in the acclimation of each new fish to the anemone. The results of these experiments are summarized below.

An unacclimated fish introduced into the tank a foot or so away from the anemone, usually approached the anemone within a few minutes and began to swim under the disk, around the column, and occasionally over the top of the disk a centimeter or more away from the tentacles. Such fish spent most of their time under the disk at this stage and sometimes were seen nibbling at the column of the anemone. Most fish seemed to "recognize" the anemone within a few minutes and swam toward it. However, in two tests, two fish failed to react noticeably to the anemone for 20 and 27 minutes, respectively. In both cases another fish was introduced directly onto the disk of the anemone where it shortly took up residence. In both tests the unreactive fish then came rather quickly toward the anemone, apparently in response to the fish already in occupancy, and began the characteristic acclimation process.

As the process proceeded, passage over the disk became more and more frequent and the "acclimating" fish moved closer and closer to the tentacles. Swimming was accomplished by a distinctive series of slow vertical undulations, in which the tail was usually held a little lower than the rest of the body. Eventually, on one of these trips over the disk, the fish would touch a tentacle or two, usually with the ventral edge of its anal fin or the lower margin of its caudal fin. Commonly this resulted in a moderate adherence of the tentacle to the fin and contraction of the tentacle. The fish then jerked itself free with a violent flexure of its body and usually raced off the disk. Not all newly introduced *Amphiprion* caused clinging upon their first contact with tentacles, but it was the general rule. However, this adherence failed to deter the fish, which nearly always returned immediately to the anemone, either under the disk or over the tentacles. In our experiments the time from initial introduction until the first physical contact between fish and anemone varied from less than 1 minute to 65 minutes.

After this initial contact the fish typically came closer and closer to the tentacles, touching them with increasing regularity. The reaction to the clinging of tentacles became less and less violent until a sudden flexure of the animal's body was the only reaction given by the fish. Mouthing or nipping of tentacles was often observed in this and later stages.

The clinging and contraction of tentacles upon contact with the fish gradually became less until it ceased altogether. At the same time the fish began to swim deeper among the tentacles, using the same slow undulating movements as when it had cruised above the disk.

Once the fish was swimming in fairly constant contact with the tentacles of the anemone, a very striking change in its behavior occurred. The general speed of swimming suddenly increased until the *Amphiprion* was dashing back and forth over the disk of the anemone, flailing unreactive tentacles aside with violent move-

ments of its body. Often the fish raced beneath the anemone and appeared in one of the folds of the disk margin, its head completely ringed in tentacles. The fish frequently maintained this vantage point for a few seconds, holding position with rapid alternate fanning movements of its pectoral fins, after which it might dash onto the disk again for another foray among the tentacles. The powerful swimming typical of this stage of the acclimation process was accomplished by rapid and strong lateral body flexures. The impression given by the swimming behavior of the fish after final acclimation was that the fish was "bathing" its entire skin surface among the tentacles.

At this point we considered the fish to be fully acclimated to the anemone, since no further clinging or tentacle contraction appeared. The time required for complete acclimation varied from about one minute to nearly three hours, with an average time of one hour.

If a fully acclimated fish was removed from the anemone and its fins or body carefully scraped with a scalpel, and then returned to the anemone, the scraped areas caused both clinging and tentacle contraction. However, fish treated in this manner did not then begin the acclimation process anew but stayed among the tentacles until clinging waned and disappeared. These fish gave evidence of discomfort from the clinging tentacles by jerking themselves free. They did not, however, rush off the disk. It would seem that treating the fish in this way partially broke down their protection.

Acclimation involves development of visual recognition of the anemone by the fish. This was demonstrated by removing fully acclimated fish from the anemone and placing them in a compartment of the observation tank separated from the anemone by a heavy glass sheet. Incoming water was introduced into the isolation compartment, flowed over and around the partition, and was discharged from the compartment containing the anemone to prevent chemical gradients from occurring which could guide the fish. In every case acclimated fish oriented strongly toward the anemone which they could see through the glass, by gathering at the glass nearest it and swimming up and down with their heads directed toward their host.

The behavior of an Amphiprion which has been resident for a time in an anemone is somewhat different from that of a newly acclimated animal. The general level of activity becomes lower though such a fish normally moves much more rapidly than an unacclimated fish. After acclimation of the fish is complete the anemone tends to become a strongly defended territory. Acclimated fishes often refuse to leave the anemone's folds even if it is lifted from the water.

EXPERIMENTS ON PROTECTION AGAINST THE HOST

In these experiments we wished to determine initially whether the presence of the fish close to but not in contact with the surface of the anemone had any *observable* effect on the anemone.

Experiment No. 1. A ½-inch I.D. plastic tube was cleaned with alcohol-ether. A small *A. percula* was slipped into the tube and shaken down it until it protruded slightly from the end. When the fish was held as close as ½ mm. from the tentacles, they showed no reaction whatever. A similar test with a control *Fundulus parvipinnis* gave identical results. Contact of a single tentacle with the *Fundulus* resulted in immediate massive discharge and clinging.

No interaction at a distance between the partners or between prey and anemone could be observed.

Next, in the hope that we might be able to identify and localize the mechanism of protection, we designed the following experiments in which direct stimulation of the anemone was employed.

Experiment No. 2. As a control, we investigated the reaction of the anemone to stimulation with a clean, flame-polished glass rod. In a number of repeated tests we saw that such stimulation caused "clumping" of the tentacles, marked adherence to the rod (discharge of nematocysts), retraction of the tentacles, and retraction of the lobe of the disk in the vicinity of the point of stimulation. Far greater mechanical stimulation and agitation of tentacles and disk by *Amphiprion* produce no noticeable response from the anemone.

Experiment No. 3. We trapped an *Amphiprion* in a beaker and held it by the lower jaw in the tips of a pair of fine-tipped forceps. Twice we drew it forcibly across the disk of the anemone, bringing it into violent contact with the tentacles. There was no discernible reaction from the anemone. The fish when released immediately entered the tentacles in a normal manner and "bathed" itself among them.

An adult *Fundulus parvipinnis* was brought into contact with the anemone and was seized in the characteristic way, involving widespread adhesion, tentacle contraction, and infolding of the disk.

An *Amphiprion percula*, trapped in a beaker and held with forceps by the jaw, was brought into contact with a large specimen of the eastern Pacific anemone, *Anthopleura xanthogrammica*. There was immediate widespread clinging so that the fish had to be pulled forcibly from the anemone.

Experiment No. 4. An *Amphiprion* was sacrificed, and we cut a cross-sectional piece of flesh, including skin, from it with a carefully cleaned scalpel. We made a similar preparation from *Fundulus*. The two preparations were placed next to each other on the disk of the anemone. The flesh from *Amphiprion* was slowly worked to the edge of the disk and cast off, while the *Fundulus* meat was enveloped and ingested. The experiment was later repeated with similar results.

Experiment No. 5. We caught an *Amphiprion*, placed it in a clean Petri dish and killed it by severing the head. We then dissected off a strip of skin, taking the greatest care to prevent contact of both surfaces of the strip with other skin surfaces. We brought this piece of freshly-removed skin into contact, *on its outer surface*, with several tentacles of the anemone. No clinging occurred except for slight adherence at the edge of the piece of skin. When the skin was brought into contact *on its inner surface*, the tentacles immediately clung strongly to it.

This experiment was repeated twice with identical results. Strips of skin from the same fish were used.

Experiment No. 6. We heated the two pieces of skin used in the preceding experiment to 90° C. for ten minutes in sea water in separate clean test tubes. The preparations were cooled. When we brought the outside surface of these heat-treated pieces into contact with tentacles, clinging immediately occurred.

Experiment No. 7. A ½-cm. cube of muscle without skin was cut from the caudal peduncle of the *Amphiprion percula*, taking great care not to bring it in contact with skin surface. It was placed on the disk of the *Stoichactis*, and was

immediately seized. The tentacles clumped around the piece and infolding of the disk margin occurred. The anemone's response differed in no discernible way from its response to *Fundulus* meat.

Experiment No. 8. Four cubes were cut from a commercial plastic sponge.

In the following tests, the "clinging reaction" of a small group of tentacles was tested. When the plastic cube was brought in contact with the tentacles, the reaction was classified arbitrarily from 0 (no clinging) to +++++ (very strong adhesion). In each test a different group of tentacles was selected. The time required for release of the cube was noted.

- a. A clean control cube: tentacles retracted; clinging 0-+; time of release < 1 second. This control was repeated several times with identical results.
- b. A similar cube of which all surfaces had been rubbed over the skin of *Amphiprion percula*: results identical with the control. This test was repeated several times with similar results.
- c. A cube rubbed over the skin of *Amphiprion frenatus*: clinging ++; retraction of tentacles, release time 20 seconds.
- d. A cube rubbed over the skin of an adult Garibaldi, *Hypsypops rubicunda* (an eastern Pacific *pomacentrid* fish): clinging +++++, released after 2 minutes 45 seconds.
- e. A cube rubbed over the skin of *Fundulus parvipinnis*: clinging +++++, released after 3 minutes 45 seconds.

Experiment No. 9. Four new cubes were cut. Two of these were rubbed over *Amphiprion percula*. One clean cube and one mucus-covered cube were heated to 100° C. for ten minutes in a dry oven and cooled.

- a. The clean control cube: tentacles retracted; clinging 0-+, time of release < 1 second. Heavy pressure caused sufficient clinging to hold the cube for as long as 4 seconds.
- b. A clean cube, heat treated: results identical with control.
- c. A mucus-covered, unheated cube: tentacular retraction; clinging 0, even under strong pressure; time of release, immediate.
- d. A mucus-covered, heat-treated cube: identical with control (a).

Identical results were obtained in a second series of tests. In this experiment we see that stronger mechanical stimulation than was used in Experiment 8 induced clinging of brief duration in a control sponge. If there was a coating of *Amphiprion percula* mucus on the sponge, clinging could not be induced even with strong pressure. But if the coating of *Amphiprion* mucus was heat-treated, its protective effect was obliterated.

The effect of heat was also shown in Experiment 6.

Experiment No. 10. On May 14, 1958, while the anemone was located in a display tank, two large groups of eggs were found attached to the rock occupied by the anemone. One patch was being guarded by an adult goby *Bathygobius saporator* (Cuvier and Valenciennes) and the other patch, which was attached in a crevice directly beneath the anemone, was guarded by two adult *Amphiprion percula*, which had been allowed to become resident in the anemone. The eggs of both species were tested for protection against the nematocysts of the anemone.

When the intact egg of the goby was touched against a tentacle, clinging occurred, and the tentacle bent into a clump with four or five other tentacles. No

movements of the disk were noted. When eggs were released in the water over the anemone and allowed to drift onto the tentacles the same effects were produced.

When these tests were repeated using *Amphiprion* eggs, the following results were obtained. Even when an egg was pressed against a tentacle with sufficient pressure to bend the tentacle no clinging resulted. Eggs dropped onto the disk through the water caused no reaction. Quite evidently *Amphiprion* eggs are as effectively protected as the adult.

EXPERIMENTS ON THE DISCHARGE OF NEMATOCYSTS FROM ISOLATED TENTACLES

Our next experiments were designed to determine whether *Amphiprion* mucus raised the threshold of nematocyst discharge. Isolated tentacles were stimulated mechanically or electrically while being observed through a dissecting microscope.

Mechanical stimulation. Pantin (1942) showed that direct mechanical stimulation of the isolated tentacle of *Anemonia sulcata* with a clean glass bead failed to cause discharge. Experiment No. 2 showed that stimulation of the *in situ* tentacle tip of *Stoichactis* with a smooth flame-polished glass rod results in clinging. Isolated tentacles of *Stoichactis* appear to be more sensitive to mechanical discharge than those of *Anemonia*. Even when the greatest care was taken in transferring a tentacle to a clean watch glass in clean sea water for isolation and stimulation, its tip very frequently stuck to the bottom of the glass for a few seconds.

In our preparations mechanical stimulation was effected by fine glass rods or by using the tip of the capillary tube of the glass electrode. Variation in the sensitivity of tentacles, the ease with which nematocysts could be mechanically discharged and our inability to deliver mechanical stimuli of precisely controlled intensity made it difficult to obtain a truly quantitative picture of threshold changes and intensity of discharge.

Our observations on the results of mechanical stimulation by the capillary tube of the electrode may be summarized as follows (discharge classified arbitrarily from 0 to +++++):

a. *Stimulation with the clean capillary tube:* An initial stimulation (light touching) at the tip of the tentacle typically produced a moderate discharge (++). Similar stimulation halfway between the tip of the tentacle and its cut base results in a lighter discharge (+). Repeated mechanical stimulation at both points results in progressively less discharge. Reduction of the discharge is not due to exhaustion of the nematocyst supply, as subsequent electrical stimulation produces massive discharge at the same points.

b. *Stimulation with the tip of the capillary tube covered with a pad of Fundulus mucus (control):* Initial light mechanical stimulation at the tip produced massive discharge (+++++) and clinging to the mucus pad.

c. *Stimulation with the tip of the capillary tube covered with mucus from Amphiprion percula:* Light stimulation of both tip and middle of the tentacle produced no discharge (0). If the tentacle was held in place by a clean glass rod and stimulated at another point by the mucus-covered capillary tube so forcibly as to deform the tentacle, the discharge of a few isolated nematocysts occurred but no clinging resulted. The effect of the pad of *Amphiprion* mucus appeared to be

limited to the area in contact with the mucus, for if an uncovered portion of the glass tube came in contact with the tentacle, discharge would occur at this point but not at points protected by the mucus pad.

d. *Stimulation with the capillary tube covered with a pad of mucus from Amphiprion frenatus*: Light touch at the tentacle tip $\rightarrow ++$. A touch at the side of the tentacle $\rightarrow 0$. This test did not appear to be significantly different in results from control (a).

e. *Stimulation with the capillary tube covered with mucus from the base of Stoichactis*: Not significantly different from control (a).

Electrical stimulation. Stimulation by faradic current was produced according to the standard method described above. The single excised tentacle, in clean sea water, was first tested for mechanically-induced discharge by light contact with the electrode at a point halfway between its tip and base. The tentacle was then given a series of three-second bursts of faradic stimulation at the same point, starting with the inductorium at its lowest setting (12).

Table I shows the threshold and intensity of discharge under different conditions in a series of tentacle preparations. Intensity of discharge was arbitrarily classified from 0 to +++++. The sensitivity of the control series in which mucus was absent varied widely. It appears that this variation in sensitivity reflects variation in the threshold of the different preparations, since after the initial mechanically-induced discharge, stimulation by pressing the electrode against the side of the tentacle elicited no further discharge in the four preparations. When a pad of *Fundulus* mucus was placed over the tip of the electrode, the very lightest mechanical contact of the electrode tip with the side of the tentacle elicited some discharge (+). Hence, if the threshold of electrically-induced discharge had been lowered by the mucus, it could not be discerned. Intensity of discharge at high levels of electrical stimulation did not appear to differ from the controls. When pads of mucus from *Amphiprion percula*, *A. frenatus* and the anemone itself were used, results did not differ significantly from the controls.

It is quite clear that the presence of mucus from the partner fish did not raise the threshold of electrically-induced discharge of nematocysts. It is also interesting to note that maximum discharge in all cases was elicited within a narrow range of inductorium setting (4-5).

DISCUSSION

The above investigations were principally directed toward understanding the physiological and behavioral mechanisms which maintain the animals in partnership and which protect the fish from the nematocysts of its host.

The reactions of unacclimated fish to the anemone were described in detail. These reactions differed considerably within our sample, but it must be remembered that the history of our nine fish was unknown to us. Some may have been free-living and some commensal with other species of anemone. However, the entire sample ultimately became acclimated to the *Stoichactis*. There is unquestionably a bond which attracts *A. percula* to this anemone and keeps the fish in it, once the acclimation process is completed. This process was first observed by Gohar (1948) who says (p. 39): "Fish of the commensal species may develop partnership with such anemones as *Discosomum giganteum* by cautiously approaching it. The

association is completed in one to a few days." Once the association is completed the bond is stonger; acclimated fish rarely wander away from the anemones, while unacclimated ones may wander all over the aquarium. In the course of the acclimation process we observed the "cautionsness" noted by Gohar. A fish touches the tentacles, often sticks at first and flees. But it *keeps coming back*, making more and more contact until no tentacular clinging occurs. We have not as yet identified those signs which attract the fish, beyond confirming Verwey's observation (1930) that the fish respond to visual cues. Since our anemone was kept in still water, part of the visual cue to an unacclimated fish may have been absent, since in nature the *Stoichactis* must be in almost constant motion in its shallow water habitat. We have not as yet investigated the possibility that specific chemical releasers from the anemone may be an important part of the bond. Tactile stimuli may also be important, for the fish appears to "seek" contact with the anemone during the acclimation process. The process of acclimation may be recognized by the action of the fish of bringing more and more of its body in contact with the host. The strength and effectiveness of stimuli from the anemone certainly affect the rate of attainment of the ultimate equilibrium between the partners, which is the consummation of the acclimation process.

The behavior of the anemone in relation to the fish was also carefully observed. Some writers have claimed that the commensals, even without contact, affect the behavior of the tentacles. Crespigny (1869) said (p. 10): ". . . a *Premnas* now passes over the anemone and immediately the tentacula become erect and diverge, while their extremities become clubby. . . ." Herre (1936), working with the symbionts used in our investigation, says (p. 167): "But when an *Amphiprion* darted in among the beautiful but dangerous tentacles, they curled away from the intrepid invader." We have never observed any such action at a distance, in spite of efforts to elicit some response by bringing an acclimated *Amphiprion*, held by its lower jaw or immobilized in a plastic tube, within a fraction of a millimeter of the host. In the former test, water currents from the fish's pectoral fins gently waved the anemone's tentacles, but no such response appeared as that described above. Even when an acclimated *Amphiprion* was dragged across the disk, no response occurred which was not attributable to mechanical disturbance; we suspect that the observations of Crespigny and Herre were merely the result of water currents.

Gohar implies that the fish in some way affect the nerve net of the anemone when he speaks of the activity of the fishes appearing (p. 38) "as if they were . . . sympathetically caressing" closed anemones so that they opened. This observation was also made by Verwey (1930). Gudger feels that such behavior involves a certain "gentle massage." This activity may be effective in bringing the anemone back to its expanded state, and if so, then the fish is affecting the neuromotor apparatus of the host. But the purposive implication is unwarranted, since the behavior of the fish is probably not very different from that when the anemone is already expanded and may be under the control of the same stimuli as those eliciting typical "acclimating activity."

We have produced other evidence that the presence of the fish may affect the neuromotor apparatus of the host, for contact by an unacclimated fish may result in localized retraction of tentacles, and, if stimulation is particularly strong, a slight infolding of the disk may occur. This reaction is similar to that elicited by contact

with prey, but is not as intense, involving briefer tentacular retraction and a weaker, more localized infolding of the disk. The frequently violent activity of the acclimated fish has no apparent effect on the anemone whatever. It is as though a physiological barrier had been set up during the acclimation process. We believe that the weak response of the anemone to an unacclimated fish indicates that the fish has not yet reached a state in which it fails to stimulate the host and that low intensity stimuli of the same nature as those received from prey are "getting through." It seems apparent that in the acclimation process, repeated contact with the anemone is necessary for the establishment of both the physiological protective barrier and the "bond of association" between the animals. We do not know the exact nature of the barrier. If one places a skinless piece of *Amphiprion* meat on the disk it is consumed, while a piece with skin attached is rejected and ultimately falls off the disk. Furthermore, if a piece of *Amphiprion* meat with skin and a piece of *Fundulus* meat with skin are placed side by side on the disk the former is rejected and the latter consumed. It appears as though the anemone "discriminated" between them. From this we are forced to conclude that a factor is present in the skin which affects the stimulus-response chain in the anemone. But the factor may do this indirectly by preventing nematocyst discharge, if, for instance, the normal feeding reactions depend upon the reception of information from receptors in the tentacles which are sensitive to bursts of nematocyst discharge, or to substances released from prey that has been "stung."

We are persuaded that the protection of the fish against its host's nematocysts does not involve a simple inability on the part of the fish to give adequate stimuli for discharge. We have shown that (1) the strongest stimulation of an isolated tentacle by a glass rod covered with *Amphiprion* mucus results in little or no discharge whereas like stimulation by a clean rod causes a burst of nematocysts; (2) when an *Amphiprion* is dragged across the disk of the host no discharge or adherence occurs; (3) the inner surface of a piece of *Amphiprion* skin sticks immediately to the tentacles, while the outer surface does not; (4) heat-treatment of the skin abolishes the protection; (5) a sponge covered with *Amphiprion* mucus will not stick to the tentacles, even when firmly pressed against them, while a clean sponge will; (6) heat-treatment of a mucus-covered sponge destroys the protection; (7) *Amphiprion* is immediately seized by another anemone (*Anthopleura*). All these observations argue for the existence of a heat-labile factor present on the outer surface of the skin of *Amphiprion*, which raises the threshold of discharge of nematocysts in the host *Stoichactis*.

What is the function of the behavioral process we call acclimation? We believe that this process, which other workers have suggested serves to change the condition of the anemone, is more probably a mechanism which changes the condition of the fish as the result of repeated contact between it and the anemone. It remains to be determined whether the fish has immunity to the nematocyst contents and whether acclimation has any relation to the maintenance of this immunity. There is a possibility, though we cannot offer conclusive proof, that acclimation may be related to changes in the mucus coat of the fish. Frequently, prior to complete acclimation, the protection of a fish is not perfect. Perhaps increasing contact with the anemone induces a greater general secretion of mucus or, specifically, more of the active principle in the mucus. Rough handling of the fish renders it susceptible

to localized stinging, which may result from "breaks" in the protective mucus coat. During the early stages of acclimation the fin tips are the sites of nearly all localized clinging. These edges are precisely the areas which are first brought carefully into contact with the anemone. Similar clinging occurs at the site of a wound in a damaged fish, but after a short time, if the wound is not great, clinging no longer occurs. This may indicate the spread of mucus over the wound, renewing the integrity of the protective coat. It would seem that the characteristic fluttering movements of *Amphiprion* when on the disk of the anemone would be particularly effective in spreading mucus over the various sharp fin edges.

Clearly there remain many unsolved problems. It appears that the protective principle in the mucus coat takes its effect locally, is fast-acting and specific. It would be of interest to determine its rate of decay and to find out whether, after cessation of contact between a tentacle and *Amphiprion* mucus, there is some effect, however brief, on the threshold of nematocyst discharge. One would wish to know a good deal more about the chemical nature of the principle and whether it is present in other fishes such as *Nomeus*, the commensal of the Portuguese Man-of-War, *Physalia*.

SUMMARY

1. The behavioral process is described whereby the fish *Amphiprion percula*, after long isolation from the anemone *Stoichactis*, effects its association with the host.

2. This process appears to involve a gradual acclimation to the host, brought about by increasing contact with the host's tentacles. This appears to effect the establishment of both the "bond" and the physiological protective barrier between the animals.

3. Evidence is presented that an active principle is present in the mucus secreted on the outer surface of the integument of *Amphiprion* which raises the threshold of mechanically-induced discharge of the host's nematocysts. This factor does not affect the threshold of electrically-induced discharge. It is fast-acting, specific in its effect and heat-labile. It is not present in the muscle of the fish.

4. After contact between the host and an acclimated commensal no feeding reactions can be observed in the anemone such as occur when similar contact is made between *Stoichactis* and prey fish or between other anemones and *Amphiprion*. It is possible that this "inhibition" of the anemone may be the result of a direct effect on the nervous system by the active principle. However, it would seem more probable that this absence of feeding reactions even on violent contact may depend upon the fact that nematocysts are not discharged. Perhaps stimuli from receptors in the tentacles sensitive to nematocyst discharge or to substances from "stung" prey are necessary for the initiation of feeding reactions.

LITERATURE CITED

- BAERENDS, G. P., 1950. Specializations in organs and movements with a releasing function. *Symposium Soc. Exp. Biol.*, 4: 337-360.
- BAERENDS, G. P., 1957. The ethological analysis of fish behavior. In: *The Physiology of Fishes*, M. E. Brown, Edit. New York, Academic Press, Inc.
- DE CRESPIGNY, C. C., 1869. Notes on the friendship existing between the Malacopterygian fish *Premnas biaculeatus* and the *Actinia crassicornis*. *Proc. Zool. Soc. London*: 248-249.

- GOHAR, H. W. F., 1948. Commensalism between fish and anemone. *Publ. Mar. Sta. Ghardaqa*, **6**: 35-44.
- GUDGER, E. W., 1947. Pomacentrid fishes symbiotic with giant sea anemones in Indo-pacific waters. *J. Asiat. Soc. Beng.*, **12**: 53-76.
- HERRE, A. W. C. T., 1936. Some habits of Amphiprion in relation to sea anemones. *Copeia*, **3**: 167-168.
- PANTIN, C. F. A., 1942. The excitation of nematocysts. *J. Exp. Biol.*, **19**: 294-310.
- SLUITER, C. P., 1888. Ein merkwürdiger Fall von Mutualismus. *Zool. Anz.*, **11**: 240-243.
- VERWEY, J., 1930. Coral reef studies, I. The symbiosis between damselfishes and sea anemones in Batavia Bay. *Trecubia*, **12**: 305-366.